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**Calicioid lichens and fungi in amber – Tracing extant lineages back to the Paleogene**

Jouko Rikkinen [a,b,*], S. Kristin L. Meinke [c], Heinrich Grabenhorst [d], Carsten Gröhn [d], Max Kobbert [d], Jörg Wunderlich [d], Alexander R. Schmidt [c,*]

[a] Finnish Museum of Natural History, P.O. Box 7, 00014 University of Helsinki, Finland

[b] Organismal and Evolutionary Biology, Faculty of Biological and Environmental Sciences, P.O. Box 65, 00014 University of Helsinki, Finland

[c] Department of Geobiology, University of Göttingen, Goldschmidtstraße 3, 37077 Go¨ttingen, Germany

[d] Amber Study Group, c/o Geological-Palaeontological Institute and Museum (CeNak) of the University of Hamburg, Bundesstraße 55, 20146 Hamburg, Germany

* Corresponding authors.

**Abstract**

Calicioid lichens and fungi are a polyphyletic grouping of tiny ascomycetes that accumulate a persistent spore mass (mazaedium) on top of their usually well-stalked ascomata ('mazaediate fungi'). In addition to extant forms, six fossils of the group were previously known from European Paleogene amber. Here we report nine new fossils and analyze the preserved features of all fossils to assess their applicability for dating molecular phylogenies. Many fossils are extremely well preserved, allowing detailed comparisons with modern taxa. SEM investigation reveals that even fine details of ascospore wall ultrastructure correspond to those seen in extant specimens. All fossils can confidently be assigned to modern genera: three to *Calicium* (Caliciaceae, Lecanoromycetes), five to *Chaenotheca* (Coniocybaceae, Coniocybomycetes), six to *Chaenothecopsis* (Mycocaliciaceae, Eurotiales), and one to *Phaeocalicium* (Mycocaliciaceae, Eurotiales). Several *Calicium* and *Chaenotheca* fossils are assignable to specific lineages within their genera, while the *Chaenothecopsis* fossils demonstrate the extent of intraspecific variation within one such lineage. Some features in the morphology of *Chaenotheca succina* nov. sp. seem to be ancestral as they have not been reported from modern species of the genus.

## 1. Introduction

Calicioid lichens and fungi constitute a diverse assemblage of ascomycetes which produce superficially similar, usually wellstalked ascomata with mature ascospores accumulating into a persistent spore mass (mazaedium) on top of the apothecium (Tibell, 1975, 1987, 1996a, 1998, 1999). The group is polyphyletic and consists of several unrelated lineages (Tibell, 1984; Wedin and Tibell, 1997; Wedin et al., 2000, 2002; Tibell, 2003; Prieto and Wedin, 2013, 2017).

Many calicioid lichens and fungi share similar habitat require- ments and for example form diverse communities on lignum and bark in temperate and boreal old-growth forests (Middelborg and Mattsson, 1987; Tibell, 1992; Selva, 1994, 2003, 2013, 2014; Rikkinen, 1995, 2003a, b, c, d; Holien, 1996, 1998; Titov, 1998, 2000, 2001; McMullin and Arsenault, 2016; Vehkaoja et al., 2017). Others are narrow specialists restricted to resinous plant exudates (Tibell and Titov, 1995; Rikkinen, 1999, 2003c, 2003d; Tuovila et al., 2011, 2013; Tuovila, 2013; Rikkinen et al., 2014, 2016; Selva and Tuovila, 2016), lichens, fungi or liverworts (Löfgren and Tibell, 1979; Muniz et al., 2013; Allen and McMullin, 2015; Suija et al., 2016), or twigs of angiosperm trees (Titov, 1994; Tibell, 1996b; Peterson and Rikkinen, 1998). Their nutritional ecologies are concurrently diverse, including both lichen-symbiotic and parasitic as well as saprophytic species. Many taxa are widely distributed and this allows the development of uniform calicioid communities over large areas. However, also many narrow endemics and interesting disjunctions are known (Tibell, 1994; Titov, 2006). Coelomycetous or hyphomycetous anamorphs are produced by a number of species in several of the genera (Tibell, 1997).

Fossilized lichens and associated microfungi have long been thought to be extremely rare. However, recent findings have shown that European Paleogene amber is a rich source of previously largely neglected fossil lichens and microfungi (Schmidt et al., 2014; Hartl et al., 2015; Kaasalainen et al., 2015; Kettunen et al., 2015, 2016, 2017). The new material has substantially increased the number of known fossils and provided interesting insights into fungal diversity and ecology (Kaasalainen et al., 2015, 2017).

Resinicolous calicioid fungi typically grow on semisolid resin and also lichenized calicioids grow on resin-producing trees and are there frequently overrun by fresh exudate. In addition to extant forms, three specimens of resinicolous *Chaenothecopsis* Vainio have been previously reported from European Paleogene amber (Rikkinen and Poinar, 2000; Tuovila et al., 2013), as well as one fossil of *Chaenotheca* (Th. Fries) Th. Fries and two of *Calicium* Persoon (Rikkinen, 2003b; Kettunen et al., 2018).

Here we describe and discuss all known fossils of calicioid lichens and fungi to date, based on new discoveries and reexamination of previously described material. Our principal aim is to critically evaluate the taxonomic affinities of these fossils and thus establish a solid basis for their future use in dating phylogenies and other research.

## 2. Material and methods

### *2.1. Origin and age of the fossil fungi*

Fossils of calicioid lichens and fungi are enclosed in a total of 15 pieces of Bitterfeld and Baltic amber. Bitterfeld amber originates from the 'Bernsteinschluff' Horizon in the upper part of the Cottbus Fm. of the Goitzsche mine, near the city of Bitterfeld, central Germany. The upper Oligocene amber-bearing sediment has an absolute age of 25.3–23.8 Ma (Knuth et al., 2002; Blumenstengel, 2004). A previous notion that Bitterfeld amber represents redeposited Eocene Baltic amber is based on the fact that there is a significant proportion of identical arthropod morphologies in amber from both localities (Weitschat, 1997). Redeposition of Baltic amber is unlikely, based on the reconstruction of the sedimentary environment of this huge amber deposit (Standke, 2008). A local reworking of pre-Chattian amber, however, has not been dispelled so far (Dunlop, 2010). In any case, Bitterfeld amber is Paleogene in age and its minimum age is ca. 24 Ma.

Baltic amber primarily derives from the marine Blue Earth layer that is predominantly exposed on the Samland Peninsula northwest of Kaliningrad (Russia). Baltic amber eroded from sediments is also found washed ashore along the coast of the Baltic Sea (Standke, 2008; Weitschat and Wichard, 2010). The absolute age of Baltic amber is still under debate. Palynological data suggest an upper Eocene (Priabonian) age (ca. 38–34 Ma) of the Blue Earth (Kosmowska-Ceranowicz et al., 1997; Standke, 1998, 2008). Fewer amounts of amber also occur in Lutetian (middle Eocene) sediments including the Lower Blue Earth and even in the upper Oligocene Lower Gestreifter Sand, leading to a possible age range of approximately 47 to 24 Ma for all Baltic amberbearing strata (Kosmowska-Ceranowicz et al., 1997; Standke, 1998, 2008; Kasinski and Kramarska, 2008).

The repository of the specimens is indicated in the description of each fossil.

*2.2. Preparation, microscopy and illustration of the fossil fungi*

For investigation, the amber pieces were ground and polished manually using a series of wet silicon carbide papers (grit from FEPA P 600 [25.8 mm particle size] to 4000 [5 mm particle size], Struers) to produce smooth surfaces for investigation. A fraction of a millimetre of amber was gradually removed from each amber piece, while frequently checking the preparation under a dissection microscope to ensure that the inclusions were not damaged (see Schmidt et al., 2012 for protocols).

Prepared amber specimens were mounted on a glass microscopic slide with the upper polished surface oriented horizontally. A drop of water was applied to the upper surface of the amber and covered with a 0.06–0.08 mm thickness glass coverslip (Menzel Inc.) to reduce light scattering from fine surface scratches and to improve optical resolution.

The amber inclusions were studied under a Carl Zeiss Stereo Discovery V8 dissection microscope and under a Carl Zeiss AxioScope A1 compound microscope, equipped with Canon 5D digital cameras. In most instances, incident and transmitted light were used simultaneously. For an enhanced illustration of the three-dimensional inclusions, the light-microscopical images are digitally stacked photomicrographic composites from up to 330 individual focal planes using the software package Helicon Focus 6.2.2.

For SEM investigation of fossil ascospores, fungal inclusions from selected calicioid specimens were partially exposed using a scalpel to remove the overlaying amber. Samples were transferred to a carbon-covered SEM mount using a wet hair from a superfine brush, sputtered with platinum/palladium (2  120 s at 20 mA, 10 nm coat thickness) using an Automatic Sputter Coater (Canemco Inc.) and examined under a field emission scanningelectron microscope (Carl Zeiss LEO 1530 Gemini).

After investigation, fragile amber pieces were fully embedded in a high-grade epoxy (Epo-Tek 301-2, Epoxy Technology) under vacuum (see Nascimbene and Silverstein, 2000 for protocols) to ensure long-term preservation of the fossils.

## 3. Systematic paleontology

Phylum Ascomycota Cavalier-Smith, 1998

Class Lecanoromycetes O.E. Eriksson et Winka, 1997

Order Caliciales Bessey, 1907

Family Caliciaceae Chevallier, 1826

Genus *Calicium* Persoon, 1794

*Calicium succini* (Caspary) Rikkinen et A.R. Schmidt, 2018 (in Kettunen et al., 2018)

Fig. 1(A, B)

1886. *Stilbum succini* – Caspary, 1886, p. 8.

1927. *Stilbites succini* (Caspary) Pia, 1927, p. 124.

Material: Baltic amber, Museum fur Naturkunde zu Berlin MB.Pb.1979/838 (Thomas Collection).

Description: Thallus crustose, episubstratic on bark fragment. Single stout ascoma rising from thallus, 480 mm high. Stipe 75–120 mm wide, brown to black; outer surface smooth and epruinose, probably with a thick hyaline envelope. Capitulum lenticular to disc-shaped, 175 mm wide. Excipulum well-developed, the edge possibly with faint pruina. Mazaedium well-developed but rather thin, protruding only slightly beyond excipulum edge. Ascospores one-septate, dark brown, ellipsoidal with a clear incision at septum, 7.5–11 x 4.5–5.5 mm. Mature ascospores with minutely uneven and at later stages fissured surface ornamentation.

Remarks: This fossil was originally described and illustrated as *Stilbum succini* by Caspary (1886) and Caspary and Klebs (1907a, b), and then transferred to the genus *Calicium* by Kettunen et al. (2018). The preserved features correspond well with those of extant species in *Calicium* Clade A (Prieto and Wedin, 2017). The size and shape of ascospores are also similar to those of amber specimen GZG.BST.27300 which contains abundant and well preserved ascomata which also reveal details of ascospore ultrastructure. While the ascomata of these two fossil specimens differ in their overall habit, it is possible that they represent the same species. The size and shape of ascomata in many extant Calicium species is extremely variable; for example, stipe length and width can vary dramatically under different ecological conditions and sometimes even within single collections (Yahr, 2015). Also the thallus may range from a thick and well-developed crust to a completely immersed thallus under different environmental conditions. Stalk and excipulum anatomy could offer additional information, but these features can only be studied from thin cross-sections that cannot be obtained from the fossil specimens.

*Calicium* cf. *succini*

Fig. 1(C–F)

Material: Bitterfeld amber, Geoscientific Collections of the University of Göttingen GZG.BST.27300 (formerly Heinrich Grabenhorst Amber Collection Li-22).

Description: 13 well-preserved ascomata and more fragmentary remains of ca. 60 additional ascomata rising from smooth bark, enclosed by several successive thin resin flows. Thallus crustose and very thin, probably largely immersed in the substrate. Ascomata slender, 490–600 (690) mm high. Stipe straight and narrow, up to 550 mm high, 55–90 mm wide, slightly thicker towards the base, dark brown; outer surface smooth and epruinose, with surface hyphae 1–2 mm wide. Capitulum 110– 210 mm wide. Excipulum well-developed, smooth and epruinose. Mazaedium well-developed, often protruding as a high column beyond excipulum edge. Ascospores one-septate, dark brown, broadly ellipsoidal with a clear incision at septum, (9) 10–12 (13.5) x 5–6 (7.5) mm. Mature ascospores with minutely uneven and at later stages fissured surface ornamentation.

SEM images (Fig. 2 (A, B)) show that the ascospore ultrastructure in the fossil is identical to that observed in several extant *Calicium* species. The outermost layer of the spore wall has warts which give the spore an irregularly reticulate surface ornamentation. Inside the fragmented outer layer is a thickened inner layer, the innermost layer of which is smooth as best seen in the septum. The incision of the septum is distinct and the septum itself thick; no septum pores or pore plugs are visible. Several cracked spores reveal cell lumina surrounded by a plasma membrane that have shrunk, became completely detached from the spore wall and are now shriveled inside their cavities. The surface ornamentation of the membrane closely mirrors that of the innermost surface of the spore wall.

Remarks: The preserved morphology of the fossil corresponds well with features that characterize extant species of *Calicium* Clade A (Prieto and Wedin, 2017), including *C. abietinum* Persoon and *C. glaucellum* Acharius. In our opinion, the fossil can be used as a minimum age constraint for this extant lineage. For a comparison between this fossil and *Calicium succini*, see under that species. There are some dark fungal structures on the thallus of the fossil which might represent pycnidium initials of the fossil *Calicium*. However, as there also is a dematiaceous hyphomycete on the same substrate, their exact affinity remains uncertain.

*Calicium* sp. A

Fig. 3

Material: Baltic amber, Geoscientific Collections of the University of Göttingen GZG.BST.27296 (formerly Volker Arnold Amber Collection 1294).

Description: Single detached ascoma, 1200 mm high. Stipe 1020 mm high, 115–140 mm wide, straight and even, not thickened towards the base, brown; outer surface smooth and epruinose, with thin hyaline envelope. Capitulum 250 mm wide and 120 mm high. Excipulum well-developed and rather thick, outer part formed by a continuation of stipe surface, the edge possibly with some very faint pruina. Mazaedium well-developed but not protruding far beyond excipulum edge. Ascospores oneseptate, dark brown, narrowly ellipsoidal with a clear incision at septum, (9) 11–15 x (5.5) 6–7 (7.5) mm. Surface of mature ascospores rough and uneven, with an ornamentation of spirally arranged ridges.

Remarks: This fossil was originally described as *Calicium* sp. by Rikkinen (2003b) and has been used as a minimum age constraint for *Calicium* (Caliciales) in dating studies (Prieto and Wedin, 2013, 2017; Beimforde et al., 2014). The preserved features of this fossil correspond well with those that characterize extant species of *Calicium* Subclade B3 (Prieto and Wedin, 2017). Extant species of this lineage (*Calicium* s. str.), including *C. viride* Persoon and *C. salicinum* Persoon, produce comparatively large and robust, well-stalked ascomata and have ascospores with a spiral ornamentation. Already in the original description (Rikkinen, 2003b) the fossilized ascospores were noted to have a rough surface ornamentation, but because the spores were deeply embedded in amber they could not be studied under high magnification. Now further polishing has brought the capitulum and some detached ascospores closer and revel that some spores appear ‘longitudinally striate’, most probably because they have spirally arranged ridges on the surface, which later becomes distorted by irregular cracks. Nevertheless, the exact affinity of the fossil still remains uncertain as also extant species of *Calicium* Subclade B1 (Prieto and Wedin, 2017), including *C. adspersum* Persoon and *C. chlorosporum* F. Wilson, have spirally arranged ridges on the ascospore surface. Extant species of this group tend to have a dense yellow (rarely brown) pruina on the mazaedium and excipulum, but this feature would not necessarily have been preserved, especially as the mazaedium of the fossil was probably reduced in size before the detached ascoma was engulfed in fresh resin and finally preserved. In any case, the fossil demonstrates that the character state of spirally striate ascospores had already evolved in the

Paleogene. Also the enigmatic *Allocalicium adaequatum* (Nylander) M. Prieto et Wedin has spirally arranged ridges on the ascospores, but it differs from the fossil in several other aspects.

Class Coniocybomycetes M. Prieto et Wedin, 2013

Order Coniocybales M. Prieto et Wedin, 2013

Family Coniocybaceae Reichenbach, 1837

Genus *Chaenotheca* (Th. Fries) Th. Fries, 1860

*Chaenotheca* sp. A

Fig. 4(A)

Material: Baltic amber, Geoscientific Collections of the University of Göttingen GZG.BST.27297 (formerly Volker Arnold Amber Collection 1285).

Description: Thallus verrucose, 100–200 mm thick, covering the bark substrate as a grayish white, glossy crust. Five 430– 480 mm high, stout ascomata arising from crustose thallus. Stipe dark brown to black, straight or slightly curved and slightly thickened towards the base, 340–360 mm high, 50–90 mm wide; outer surface smooth and epruinose, surface hyphae not clearly visible (possibly due to the presence of a hyaline envelope). Capitulum 100–120 mm wide. Excipulum well developed, cupshaped, smooth and epruinose. Mazaedium not protruding far beyond excipulum edge. Ascospores non-septate, spherical or slightly ellipsoidal, (3.5) 5–7 mm, coarsely ornamented when mature.

Remarks: This fossil was originally described as *Chaenotheca* sp. by Rikkinen (2003b) and has since been used as a minimum age constraint for *Chaenotheca* (Coniocybales) in dating studies (Prieto and Wedin, 2013; Beimforde et al., 2014). As many extant *Chaenotheca* species are morphologically plastic and identifying them requires examination of ascus and photobiont characteristics, the fossil cannot be assigned to any modern species. However, some of its preserved features correspond remarkably well with those of the extant *Chaenotheca ferruginea* (Turner ex Smith) Migula. Both fungi have relatively robust ascomata with a well-developed, cup-shaped excipulum. They also have larger ascospores than most other *Chaenotheca* species, with a similar surface ornamentation. *Chaenotheca ferruginea* can produce a thick thallus similar to that of the fossil, and it commonly grows on conifer bark, often around fresh resin flows and sometimes even on solidified resin (Rikkinen, 2003a). Nevertheless, over its extensive range

*Chaenotheca ferruginea* is variable and may actually represent a complex of several taxa (Rikkinen, 2003a). Its exact affinities within the genus are not known, but it seems to belong to the same lineage as *Chaenotheca hygrophila* Tibell (Suija et al., 2016), another species that has relatively robust ascomata and a well-developed excipulum. This fossil can probably be used as a minimum age constraint for this extant lineage after a more comprehensive assessment of intrageneric relationships within *Chaenotheca* is available.

*Chaenotheca* sp. B

Fig. 4(B–E)

Material: Baltic amber, Kobbert P196 (Max Kobbert Amber Collection, Munster, Germany) (Fig. 4(B, C)); Baltic amber, Geological-Palaeontological Institute (CeNak) of the University of Hamburg GPIH 4934 (formerly Carsten Gröhn Amber Collection 6463; Fig. 4(D, E)).

Description:

Kobbert P196: Thallus coarsely granular, covering the bark substrate as an uneven crust. Nine ascomata arising from the thallus, 130–350 mm high. Stipe brown and 25–50 mm wide, straight or slightly curved, not markedly thickened at the base, with smooth and epruinose surface, composed of periclinally arranged hyphae 1.0–3.0 mm wide. Capitulum light brown, 135–185 mm wide. Excipulum poorly developed, formed as a continuation of surface hyphae of the stipe, not distinct in mature apothecia with well-developed mazaedia. Ascospores non-septate, subspherical, (2.5) 3–4 (4.5) mm, with smooth surface.

GPIH 4934: Thallus granular, covering on surface of old resin flow as an uneven crust. Seven mature ascomata and five initials 105–590 mm high, arising from crustose thallus. Stipe dark brown to black, (20) 40–60 mm wide, slightly flexuous and thickening towards the base, with smooth and epruinose surface, composed of periclinally arranged hyphae 1.0–4.5 mm wide. Capitulum brown, 120–270 mm wide. Excipulum obconical, smooth and epruinose, not distinct in mature apothecia with well-developed mazaedia. Ascospores non-septate, spherical, (2) 2.5–4 mm, with smooth surface.

Remarks: On the basis of thallus structure, habit of ascomata and shape and size of ascospores, these two fossils seem to belong to the same species. However, confirming this would require a detailed examination of ascus and photobiont characteristics, which are not accessible in the fossils. In any case, on the basis of preserved features they correspond perfectly with several modern species including *Chaenotheca trichialis* (Acharius) Th. Fries. This extant species typically

has a granular to minutely squamulose thallus closely resembling the thallus fragments preserved in specimen Kobbert P196. It commonly grows on conifer bark, frequently around fresh resin flows and sometimes even on solidified resin (Rikkinen, 2003a). Phylogenetic relationships within the genus *Chaenotheca* are poorly understood, but *C. trichialis*, *C. xyloxena* Nadvornık, and *C. cinerea* (Persoon) Tibell seem to belong to the same lineage, distinct from that of *C. ferruginea* and its close relatives (Tibell, 2002; Prieto et al., 2013; Suija et al., 2016). These two fossils can probably be used as a minimum age constraint for this extant lineage after a more comprehensive assessment of intrageneric relationships within *Chaenotheca* becomes available.

*Chaenotheca* sp. C

Fig. 4(F)

Material: Bitterfeld amber, Geoscientific Collections of the University of Göttingen GZG.BST.21961 (formerly Heinrich Grabenhorst Amber Collection Le-75).

Description: Thallus thin, hardly visible on surface of old resin flow. Single 520 mm high, slender ascoma arising from crustose thallus. Stipe dark brown to black, 440 mm high, 40–70 mm wide, slightly flexuous and thickening towards the base, with smooth and epruinose surface, composed of periclinally arranged hyphae 1.8–2.5 mm wide. Capitulum 90 mm wide. Excipulum formed as a continuation of the stalk tissue and only visible as an indistinct collar at the base of the capitulum. Mazaedium compact due to the loss of peripheral parts of a previously more voluminous spore mass. Ascospores non-septate and spherical, 3–5 mm, with smooth surface.

Remarks: On the basis of the single mature ascoma preserved it is not possible to determine with any certainty whether it belongs to the same taxon as the two previously described fossils or represents a different species. It differs from the two other fossils (Kobbert P196 and GPIH 4934) in having an indistinct excipulum and compact mazaedium, but these features are almost certainly modifications caused by a loss of mazaedial matter prior to preservation, probably after the piece of dry resin supporting the fungus had become detached from its original site but before it was submerged into fresh resin and preserved together with bark fragments and other loose debris.

*Chaenotheca succina* Rikkinen et A.R. Schmidt, nov. sp.

Fig. 5

Derivation of the name: The epithet refers to the occurrence of the fossil in amber (Latin: succinum).

Holotype: Geological-Palaeontological Institute (CeNak) of the University of Hamburg GPIH 4933 (formerly Carsten Gröhn Amber Collection 5377). The ascoma illustrated in Fig. 5(B) represents the holotype.

MycoBank: MB 824675.

Type Locality and Horizon: Priabonian (34–38 Ma) amberbearing Blue Earth layers, Samland Peninsula near Kaliningrad, Russia.

Diagnosis: *Chaenotheca succina* differs from other *Chaenotheca* species in having the stipe surface composed of intertwining hyphae, persisting paraphyses in the mazaedium, and a continuous thin outer layer in the ascospores.

Description: Thallus crustose, covering bark or lignum. Fifteen (470) 820–930 mm high, slender ascomata arising from crustose thallus. Stipe dark brown, (410) 690–820 mm high, (50) 60– 100 mm wide, curved or slightly flexuous, not markedly thickened at the base, with smooth and epruinose surface, composed of periclinally arranged, but intertwining hyphae 1.0–2.5 mm wide. Capitulum 60–150 mm wide, dark brown. Excipulum obconical, smooth and epruinose, only covering the lower section of the capitulum and not distinct in mature apothecia with voluminose mazaedia. Mazaedium well-developed, supported by persisting paraphyses, 1.5–2.5 (4) mm thick and protruding far beyond excipulum edge. Ascospores non-septate, spherical to slightly ellipsoidal, (2.5) 3–5 mm, with smooth surface.

SEM images (Fig. 2(C, D)) show that mature ascospores have a very thin continuous outer layer, enclosing a thickened inner layer of coarse spore wall material. In several spores the lumina surrounded by a plasma membrane shrunk during preservation, became completely detached from the spore wall and are now shriveled inside their cavities. The surface ornamentation of the membrane mirrors that of the innermost surface of the spore wall.

Remarks: *Chaenotheca succina* nov. sp. differs from all other known *Chaenotheca* species in having a stalk surface of intertwining hyphae, persisting paraphyses in the mazaedium, and a continuous thin outer layer in its ascospores. These features are sufficient for distinguishing a new fossil species in Paleogene amber. All three features may represent ancestral character states that have not been preserved in modern species of the genus. There are numerous coelomycetous anamorphs (pycnidia) 20– 80 mm in diameter on the substrate, which might belong to the fossil lichen. However, it is also possible that they belonged to some other ascomycete that grew on the same substrate or even on the *Chaenotheca* thallus. For example, several species of Chaenothecopsis that parasitize modern *Chaenotheca* species regularly produce coelomycetous

anamorphs (Tibell, 1993a, 1997). Pycnidia are not usually seen in field specimens of modern *Chaenotheca* species (Tibell, 1980, 1999), but some species have been observed to produce coelomycetous anamorphs in culture (Tibell, 1993b, 1997).

Class Eurotiomycetes O.E. Eriksson et Winka, 1997
Order Mycocaliciales Tibell et Wedin, 2000
Family Mycocaliciaceae A.F.W. Schmidt, 1970
Genus *Chaenothecopsis* Vainio, 1927

*Chaenothecopsis bitterfeldensis* Rikkinen et Poinar, 2000
Fig. 6 (A, H)

Material: Bitterfeld amber, Geoscientific Collections of the University of Göttingen GZG.BST.21970 (formerly George O. Poinar Amber Collection AF 9-26).

Description: Mycelium extensive on old resin surface, partly superficial and penetrating into the substrate. Hyphae 1–2 mm in diameter. Numerous (> 20) ascomata arising from mycelium, 460– 750 mm high. Stipe black, often slightly flexuous, up to 550 mm high, 45–80 mm wide, with smooth and epruinose surface consisting of interwoven hyphae. Capitulum up to 210 mm wide. Excipulum less than 10 mm thick, formed as a continuation of the stalk tissue and mainly consisting of periclinally arranged hyphae. Ascospores pale brown, narrowly ellipsoidal to cylindrical, 1- septate, (6) 10–15 x 4–5 (6) mm, with smooth surface.

Remarks: This fossil was originally described by Rikkinen and Poinar (2000). The affinities to extant *Chaenothecopsis* species were discussed in Tuovila et al. (2014). The preserved ascomata represent all developmental stages from small initials to mature ascomata with rather well-developed mazaedia. Many mature, detached ascospores occur in the surrounding amber matrix. Their preservation is excellent, with septa clearly visible. The amber even contains spores that had germinated prior to preservation, showing different stages of development from spore swelling and germ tube formation to hyphal elongation. Several ascomata emerge from a dense hyphal mat and also their substrate is completely infested with hyphae, often with spirally twisted regions and irregular hyphal walls.

*Chaenothecopsis bitterfeldensis* closely mirrors several extant species. For example, except for being slightly larger, the ascospores of the fossil are identical to those of *C. sitchensis* and other

resinicolous taxa of *Chaenothecopsis* Group D (Tuovila et al., 2014). In our opinion, this fossil can be used as a minimum age constraint for resinicolous species in that extant lineage.

*Chaenothecopsis* cf. *bitterfeldensis*

Fig. 6(B–G, I, J)

Material: Baltic amber, Geoscientific Collections of the University of Göttingen GZG.BST.27286 (formerly Jörg Wunderlich Amber Collection F1178/BB) (Fig. 6(B, C, I)); Bitterfeld amber, Geoscientific Collections of the University of Göttingen GZG.BST.27285 (formerly Heinrich Grabenhorst Amber Collection Li-83) (Tuovila et al., 2013: Fig. 7); Baltic amber, Geoscientific Collections of the University of Göttingen GZG.BST.21972 (formerly Jörg Wunderlich Amber Collection F2026/BB) (Fig. 6(D)); Bitterfeld amber, Geoscientific Collections of the University of Göttingen GZG.BST.21962 (formerly Heinrich Grabenhorst Amber Collection Mi-55) (Fig. 6(E)); Bitterfeld amber, Geoscientific Collections of the University of Göttingen GZG.BST.21971 (formerly Heinrich Grabenhorst Amber Collection Le-127) (Fig. 6(F, G, J)).

Description:

GZG.BST.27286: Extensive mycelium on old resin surface, partly superficial and penetrating into the substrate. Numerous (> 15) robust ascomata arising from mycelium, up to 1840 mm high when mature; developing ascoma first with sharply pointed apices. Stipe brown, straight, 50–140 mm wide, with smooth and epruinose surface, consisting of partly intertwined hyphae (fine details not visible due to thin film of air around the inclusions). Capitulum 260–400 mm wide and 120–200 mm high, with welldeveloped excipulum of partly intertwined hyphae and mature ascospores accumulating as a mazaedial layer on top of epithecium. Ascospores narrowly ellipsoidal to cylindrical, one-septate, 8–11 x 3–4 mm, with smooth surface.

GZG.BST.27285: Extensive mycelium on old resin surface, partly superficial and penetrating into the substrate. Numerous (> 50) ascomata arising from mycelium represent different developmental stages from young initials to mature and senescent ascomata. Individual ascomata 250–1300 mm high, forming stacks of up to three ascomata of different ages by proliferating and branching. Stipe dark brown, 30–80 mm wide, smooth and consisting of partly intertwined hyphae. Capitulum with welldeveloped excipulum of partly intertwined hyphae and mature ascospores accumulating as a mazaedial layer on top of epithecium. Ascospores narrowly ellipsoidal to cylindrical, one-septate, 9–10.5 x 3.5–4.5 mm, with smooth surface.

GZG.BST.21972: Mycelium poorly preserved, mainly inside a weathered and severely degraded resin surface, which also contains organic debris, several different hyphal morphologies, and possible coelomycetous anamorphs or microsclerotia. Numerous (> 40) ascomata arising from the substrate, representing different developmental stages from young initials to senescent ascomata. Individual ascomata 130–550 mm high, commonly forming stacks of two ascomata via proliferation through the capitulum. Stipe dark brown, straight or sometimes flexuous, rarely branching, 90–420 mm high, 22–80 mm wide, smooth and consisting of partly intertwined hyphae 1.5–3 mm wide. Capitulum up to 270 mm wide, with well-developed excipulum of partly intertwined hyphae and mature ascospores accumulating as a mazaedial layer on top of epithecium. Ascospores brown, narrowly ellipsoidal, one-septate, (6) 9–10.5 (12) x 3–5 (6) mm, with smooth surface.

GZG.BST.21962: Nine ascomata caught in a spider web together with portions of the mycelium. Individual ascomata 230–485 mm high. Stipe black, straight or occasionally flexuous, 200–360 mm high, 20–40 mm wide, surface with densely intertwining, 1.5– 3 mm wide hyphae. Capitulum dark, 100–150 mm in diameter, with a well-developed excipulum of intertwined hyphae, and later subspheric with a rather extensive mazaedium of mature ascospores. Ascospores brown, ellipsoidal, one-septate, 6–7 (9) x 2–3 (4) mm, surface appearing smooth under the light microscope. SEM images confirm that the details of ascospore ultrastructure in this fossil are comparable to those of extant *Chaenothecopsis* species and more or less identical to those described for specimen GZG.BST.21971.

GZG.BST.21971: Ten ascomata and one initial that were detached from their original substrate simultaneously and moved to the current position by flowing resin. The surrounding amber contains an extensive, loose mycelium of hyphae of 1–2 mm diameter with twisted regions, closely corresponding to those typically produced by resinicolous Chaenothecopsis species. In addition, six fragmentary preserved ascomata (mainly capitula) are located close to the polished surface of the amber piece. Individual ascomata 200–450 mm high, representing immature, mature and senescent stages. Stipe dark brown, straight or curved, up to 340 mm high, 30–110 mm wide, surface with densely intertwining, 1.5–3 mm wide hyphae. Capitulum dark, 80–220 mm in diameter, with well-developed excipulum of intertwined hyphae, and mature ascospores accumulating as a mazaedial layer on top of epithecium. Ascospores brown, ellipsoidal, oneseptate, (5) 8–10 (12.5) x 2–3 (5) mm, surface appearing smooth under the light microscope.

SEM images (Fig. 2(E, F)) show that details of ascospore wall ultrastructure in the fossil are similar to those observed in extant *Chaenothecopsis* species. The spore wall has a very thin and homogenous outer layer enclosing a much thicker and more irregular inner layer. The outer membrane still covers the surface of the spore in mature spores. The incision of the septum is indistinct, but the spore surface has a weak ornamentation of low ridges, reflecting irregularities in the thickness of the inner wall layer. The coarse, uneven structure of the inner wall layer is seen in several cracked spores, which also reveal the presence of a smooth plasma membrane inside the wall. They also allow views of the septum, and in one case point towards the existence of a central pore that typically penetrates the septum of ascomycete ascospores.

Remarks: The fossil GZG.BST.27286 (Baltic amber) was first reported by Tuovila et al. (2013) as *Chaenothecopsis* cf. *bitterfeldensis*, and used as a minimum age constraint for *Chaenothecopsis* (Mycocaliciales) by Beimforde et al. (2014). The fossil GZG.BST.27285 (Bitterfeld amber) was likewise first reported by Tuovila et al. (2013) as *Chaenothecopsis* sp. While these five fossils show some differences in the size and shape of ascomata, they are however remarkably similar both to each other and to *Chaenothecopsis bitterfeldensis*. In fact, even on the basis of all features preserved, the fossils cannot be confidently distinguished from all extant species in this lineage. Together with the last mentioned taxa, they can be confidently placed in *Chaenothecopsis* Group D (Tuovila et al., 2014). Collectively they provide a comprehensive view of morphological variation in this phylogenetic lineage, and confirm a remarkable degree of stasis in their morphology and substrate ecology.

Genus *Phaeocalicium* A.F.W. Schmidt, 1970

*Phaeocalicium* sp.

Fig. 7

Material: Bitterfeld amber, Geoscientific Collections of the University of Göttingen GZG.BST.21960 (formerly Heinrich Grabenhorst Amber Collection Le-10).

Description: Mycelium not seen, submerged into unknown substrate. Two tiny ascomata arising from the substrate, 120 and 180 mm high. Stipe dark brown, slightly bent, 100 and 120 mm high, 20–25 mm wide, with smooth surface possibly consisting of periclinally arranged hyphae (hyphal structure not clearly visible due to thin film of air around the inclusions). Capitulum obovoid or strongly compressed, 80 x 60 and 70 x 35 mm wide. Excipulum at least partially

consisting of periclinally arranged hyphae. Ascospores pale brown, ellipsoidal, non-septate to two-septate, 3–4.5 x 1.5–2 mm, with smooth surface.

Remarks: This minute fungus is placed in the genus Phaeocalicium mainly on the basis of the distinctly compressed capitula of two preserved ascomata not known from other genera of Mycocaliciales or other extant calicioids. All extant species of *Phaeocalicium* have larger ascospores that those of this fossil, typically being in the size range of 10–18 x 4–6 mm. However, measurement from the fossil are only based on a few ascospores attached to the epithecium surface of one possibly semi-mature ascoma, and may thus not be representative of actual variation in spore size.

**4. Discussion**

Extant species of resinicolous calicioid fungi colonize semisolid resin and invariably produce their ascomata on hardened resin or resin-impregnated wood and bark. Also many calicioid lichens frequently grow around resin-producing cankers and sometimes produce their ascomata on hardened conifer resin. In these locations their tiny ascomata are easily trapped by fresh resin flows and can be preserved as fossils if the resin happens to turn into amber. Indeed, altogether six fossil specimens of resinicolous *Chaenothecopsis*, three specimens of *Calicium*, five specimens of *Chaenotheca*, and one specimen of *Phaeocalicium* are now known from Baltic and Bitterfeld ambers (Rikkinen and Poinar, 2000; Rikkinen, 2003b; Tuovila et al., 2013; Kettunen et al., 2018; this study). This is quite remarkable considering the overall scarcity of lichens and associated fungi among the diverse biota that have been described so far from amber (Kaasalainen et al., 2015, 2017; Hartl et al., 2015; Kettunen et al., 2015, 2017).

Calicioids include both lichen-symbiotic, parasitic and saprotrophic species. The polyphyletic nature of the group was already demonstrated by Tibell (1984) on morphological grounds and has since then been confirmed in many molecular studies (Wedin and Tibell, 1997; Tibell and Wedin, 2000; Wedin et al., 2000, 2002; Tibell, 2003, 2006; Hibbett et al., 2007; Prieto et al., 2013; Prieto and Wedin, 2013, 2017; Beimforde et al., 2014; van den Broeck et al., 2017).

Many of the fossil calicioids found from Paleogene ambers are resinicolous fungi of the genus *Chaenothecopsis*. The first fossil from Bitterfeld amber (GZG.BST.21970) was described as *Chaenothecopsis bitterfeldensis* by Rikkinen and Poinar (2000). The ascomata of the type specimen represent different developmental stages from small initials to mature ascomata, some with a welldeveloped spore mass on top of the capitulum. The preservation of ascospores is excellent,

and as some of the spores had germinated prior to preservation, they reveal different stages of hyphal development. The ascomata arise from a superficial hyphal mat, but also the original resin substrate is regularly infested with hyphae, demonstrating that the fungi exploited the resin substrate as a food source. In its overall morphology and anatomical details, *C. bitterfeldensis* closely resembles its extant relatives in *Chaenothecopsis* Group D (Tuovila et al., 2014). Also the ascospores of the fossils are identical to those produced by extant taxa. All the other five *Chaenothecopsis* fossils from European amber are very similar to both *C. bitterfeldensis* and to each other, and most probably belong to the same species or lineage. The range of morphological variation captured by the six fossils is comparable to that seen in some extant species. SEM analysis of ascospores from two fossils confirms that even at the ultrastructural level the fossils correspond exactly to extant species.

The first fossil of a calicioid lichen from Baltic amber (MB.Pb.1979/838) was already described by Robert Caspary in 1886 as *Stilbum succini*, and only recently transferred to *Calicium* by Kettunen et al. (2018). The ascospores of this fossil and another specimen from Bitterfeld amber (GZG.BST.27300) support the placement of these fossils into *Calicium* clade A (Prieto and Wedin, 2017). Again, also the SEM analysis of ascospores gives strong support to this assignment. The third *Calicium* specimen from Baltic amber (GZG.BST.27296) does not belong to the same subclade and cannot be safely assigned to any extant group as species in more than one extant lineages (subclades B1 and B3; Prieto and Wedin, 2017) produce similar ascospores.

Four of the five *Chaenotheca* fossils from European ambers are very similar to their extant relatives, but many modern *Chaenotheca* species are notoriously plastic and their accurate identification requires careful examination of features that cannot be seen in the fossils. As we still only have limited information on intrageneric relationships within the genus *Chaenotheca*, the fossils cannot be safely assigned to any specific lineages within their genus. The fifth *Chaenotheca* fossil (GPIH 4933) differs both from the other fossils and extant species in several anatomical details; it is thus described here as a new fossil species, *Chaenotheca succina*. The features that characterize this new fossil taxon seem to represent ancestral features since they have not yet been reported from modern species of the genus.

Finally, the overall morphology of the two ascomata preserved in the single *Phaeocalicium* fossil are similar to those of extant species of the genus, but they do not reveal anatomical details or information of ascospore ultrastructure. Intrageneric relationships within the genus *Phaeocalicium* have not yet been studied with DNA methods (Tibell, 1996b; Titov, 2006). Thus,

very little can be presently said about the exact affinities of the fossil, except that the compressed capitulum, a diagnostic feature also shared by several extant species, had already evolved in the Paleogene.

As a whole, the new findings described here demonstrate that the characteristic morphologies of *Calicium*, *Chaenotheca*, *Chaenothecopsis* and *Phaeocalicium* ascomata had already evolved by the Paleogene and have since remained unchanged. All these fungi have conservatively maintained morphological features that are relevant for their spore-saving strategy and the long-term maintenance of specialized niches in forest habitats (Tibell, 1984, 1994; Rikkinen, 1995, 2003a,b,c,d). To date, three of the fossils have been used as minimum age constraints for dating phylogenies of Ascomycota (Prieto and Wedin, 2013, 2017; Beimforde et al., 2014). Our new results emphasize the potential value of these fossils for dating phylogenies. However, adequate sampling of extant taxa must first be performed to provide robust hypotheses of intrageneric relationships within *Chaenotheca*, *Phaeocalicium* and the other calicioid genera.

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**FIGURES**

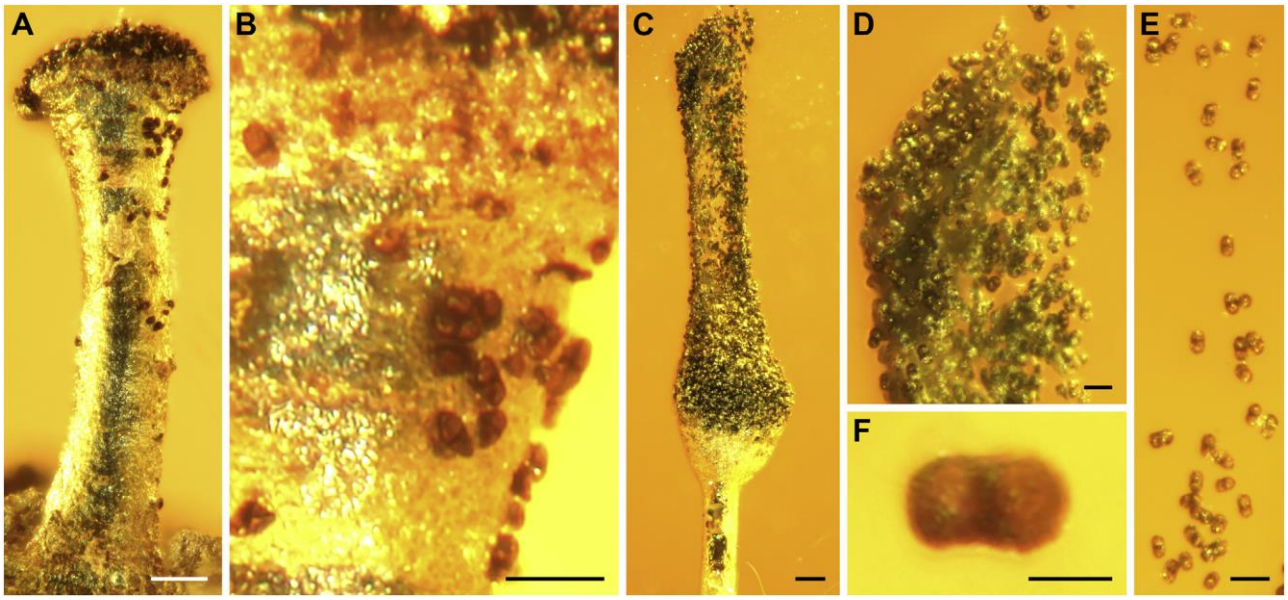


Fig. 1. Fossil *Calicium* specimens from European Paleogene amber. A, B. *Calicium succini* from Baltic amber (MB.Pb.1979/838). A: Mature ascoma, note the detached ascospores on the stipe surface; B: Detail of one-septate ascospores. C–F. *Calicium* cf. *succini* from Bitterfeld amber (GZG.BST.27300). C: Capitulum with voluminous mazaedium; D: Ascospores released from mazaedium; E: Detached ascospores; F: Mature one-septate ascospore. Scale bars: 50 mm (A, C), 20 mm (B, D, E), 5 mm (F).

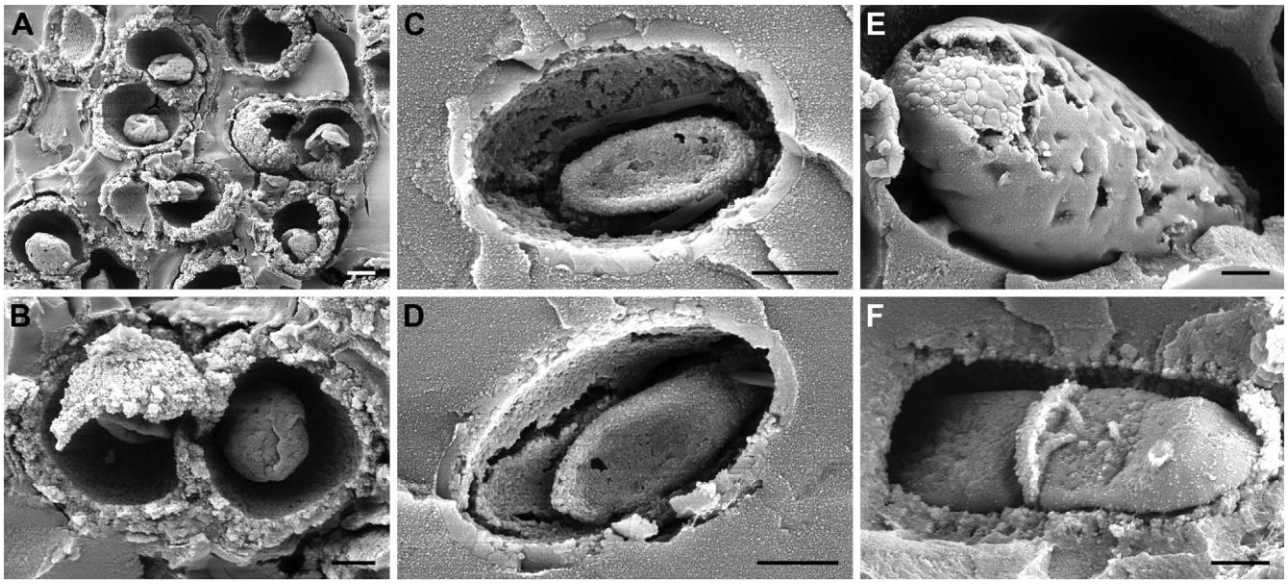


Fig. 2. SEM images revealing ascospore ultrastructure of calicioid fungi preserved in European Paleogene amber. A, B. *Calicium* cf. *succini* from Bitterfeld amber (GZG.BST.27300); cracked one-septate ascospores showing structural details of spore wall. C, D. *Chaenotheca succina* nov. sp. from Baltic amber (GPIH 4933); cracked nonseptate ascospores showing structural details of spore wall. E, F. *Chaenothecopsis* cf. *bitterfeldensis* from Bitterfeld amber (GZG.BST.21971); cracked non-septate ascospores showing structural details of spore wall. Scale bars: 2 mm (A), 1 mm (B–F).

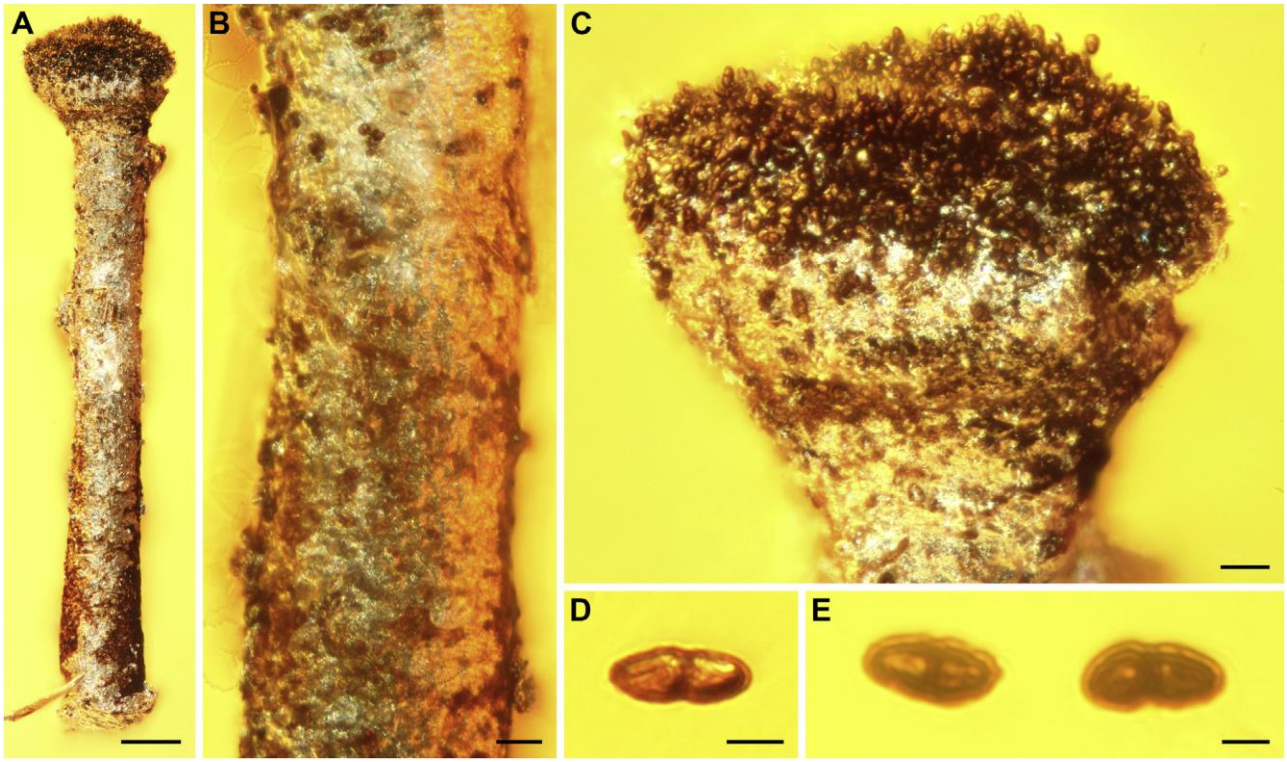


Fig. 3. Fossil *Calicium* sp. A from Baltic amber (GZG.BST.27296). A. Mature ascoma. B. Detail of stipe surface. C. Detail of exciple and mazaedium. D, E. Mature one-septate ascospores. Scale bars: 100 mm (A), 20 mm (B, C), 5 mm (D, E).

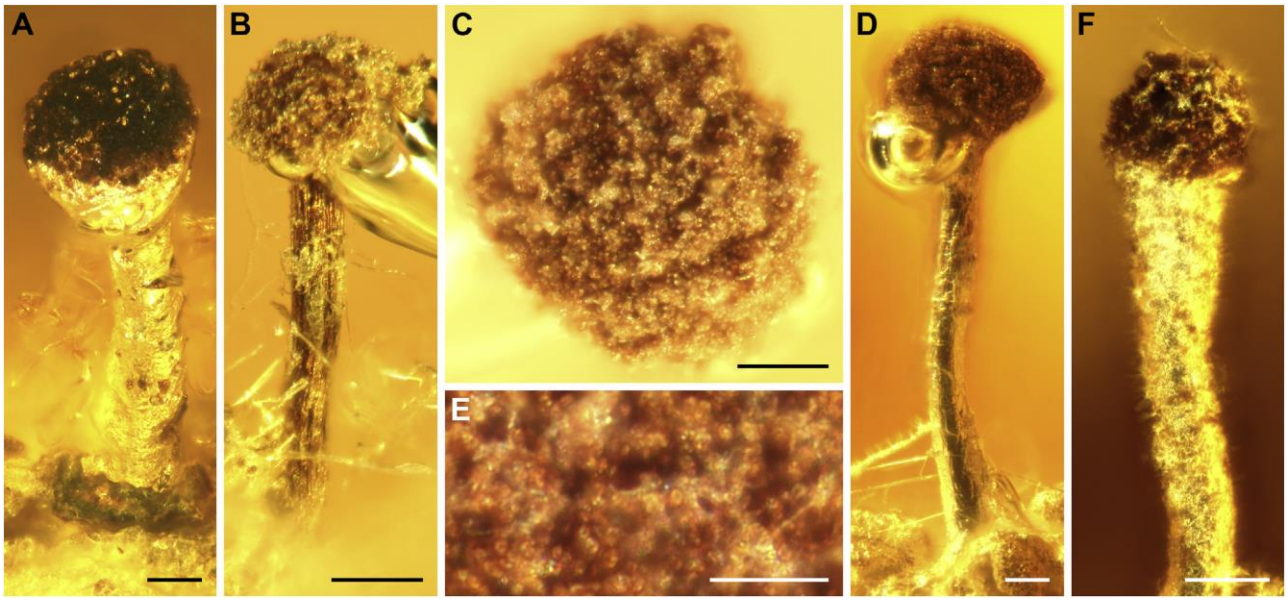


Fig. 4. Fossil *Chaenotheca* specimens from European Paleogene amber. A. *Chaenotheca* sp. A from Baltic amber (GZG.BST.27297). B, C. *Chaenotheca* sp. B from Baltic amber (Kobbert P196). D, E. *Chaenotheca* sp. B. from Baltic amber (GPIH 4934). F. *Chaenotheca* sp. C from Bitterfeld amber (GZG.BST.21961). Scale bars: 50 mm (A–D, F), 20 mm (E).

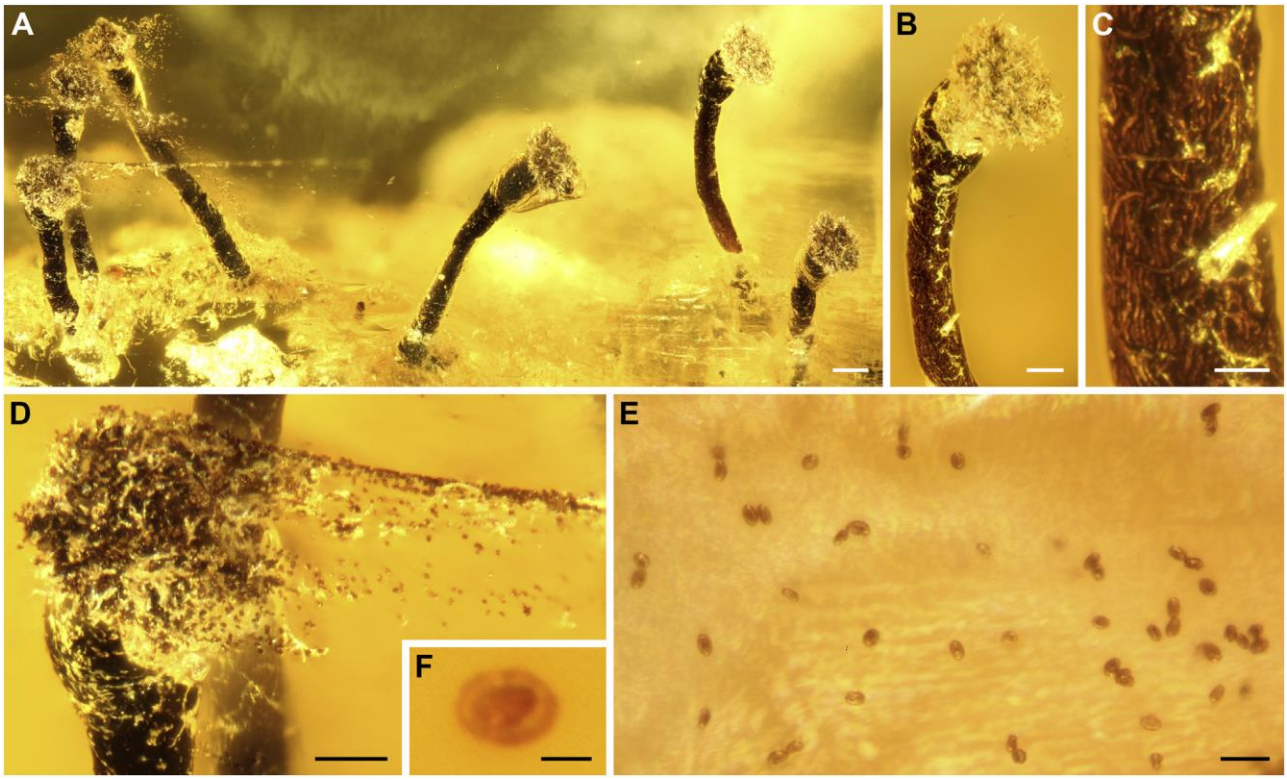


Fig. 5. *Chaenotheca succina* nov. sp. from Baltic amber (GPIH 4933). The ascoma illustrated in B represents the holotype. A. Mature ascomata. B. Exciple and mazaedium. C. Stipe surface. D. Detail of mazaedium. E. Detached ascospores. F. Mature none-septate ascospore. Scale bars: 100 mm (A), 50 mm (B–D), 10 mm (E), 2 mm (F).

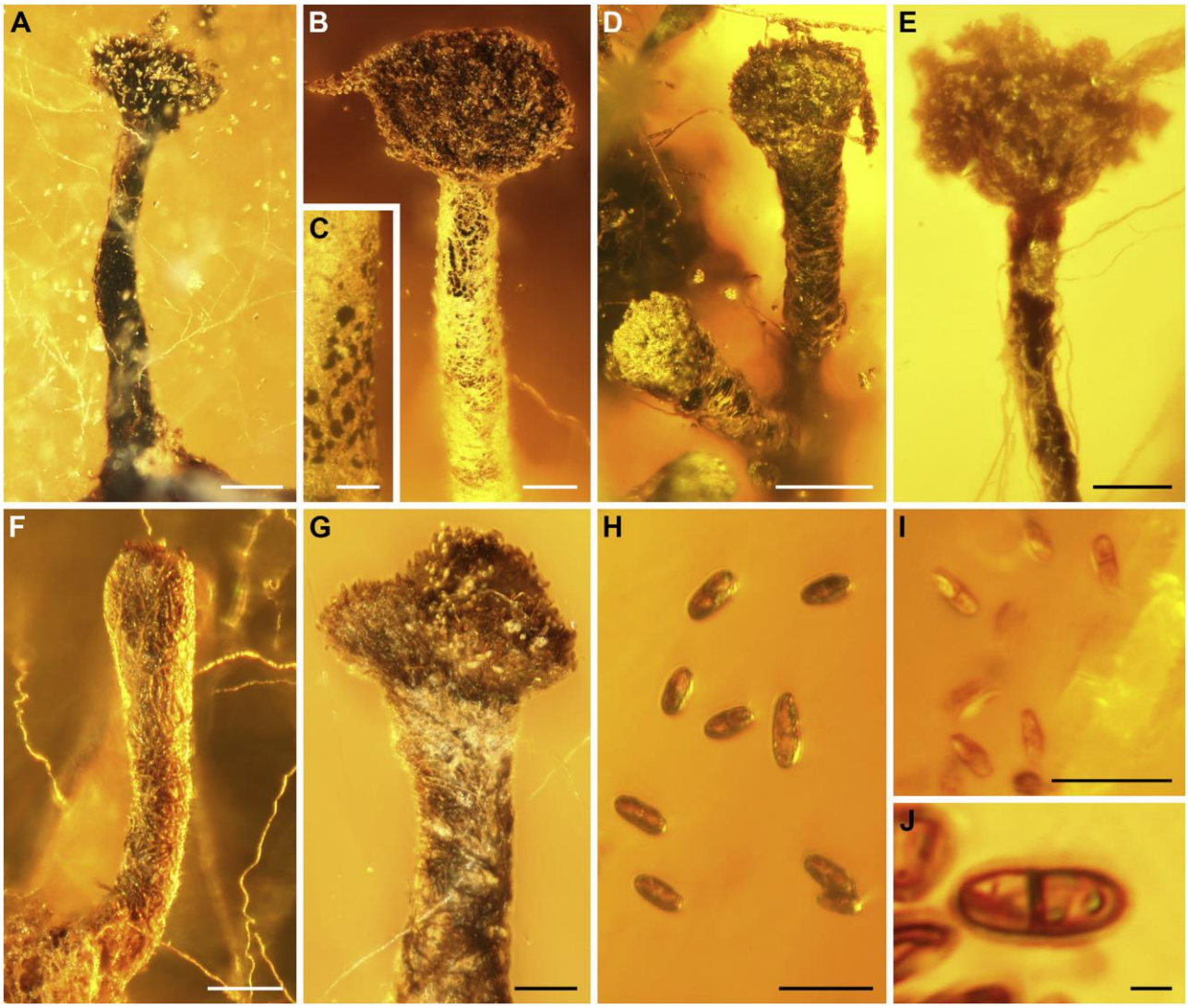


Fig. 6. Fossil *Chaenothecopsis* specimens from European Paleogene amber. A, H. *Chaenothecopsis bitterfeldensis* from Bitterfeld amber (GZG.BST.21970), showing mature ascoma (A) and mature ascospores (H). B, C, I. *Chaenothecopsis* cf. *bitterfeldensis* from Baltic amber (GZG.BST.27286), showing mature ascoma (B), stipe surface (C), and mature ascospores (I). D. *Chaenothecopsis* cf. *bitterfeldensis* from Baltic amber (GZG.BST.21972), showing two young ascomata. E. *Chaenothecopsis* cf. *bitterfeldensis* from Bitterfeld amber (GZG.BST.21962), showing mature ascoma. F, G, J. *Chaenothecopsis* cf. *bitterfeldensis* from Bitterfeld amber (GZG.BST.21971), showing immature ascoma (F), mature ascoma (G), and mature ascospore (J). Scale bars: 100 mm (A, B, D), 50 mm (C, E–G), 20 mm (H, I), 2 mm (J).

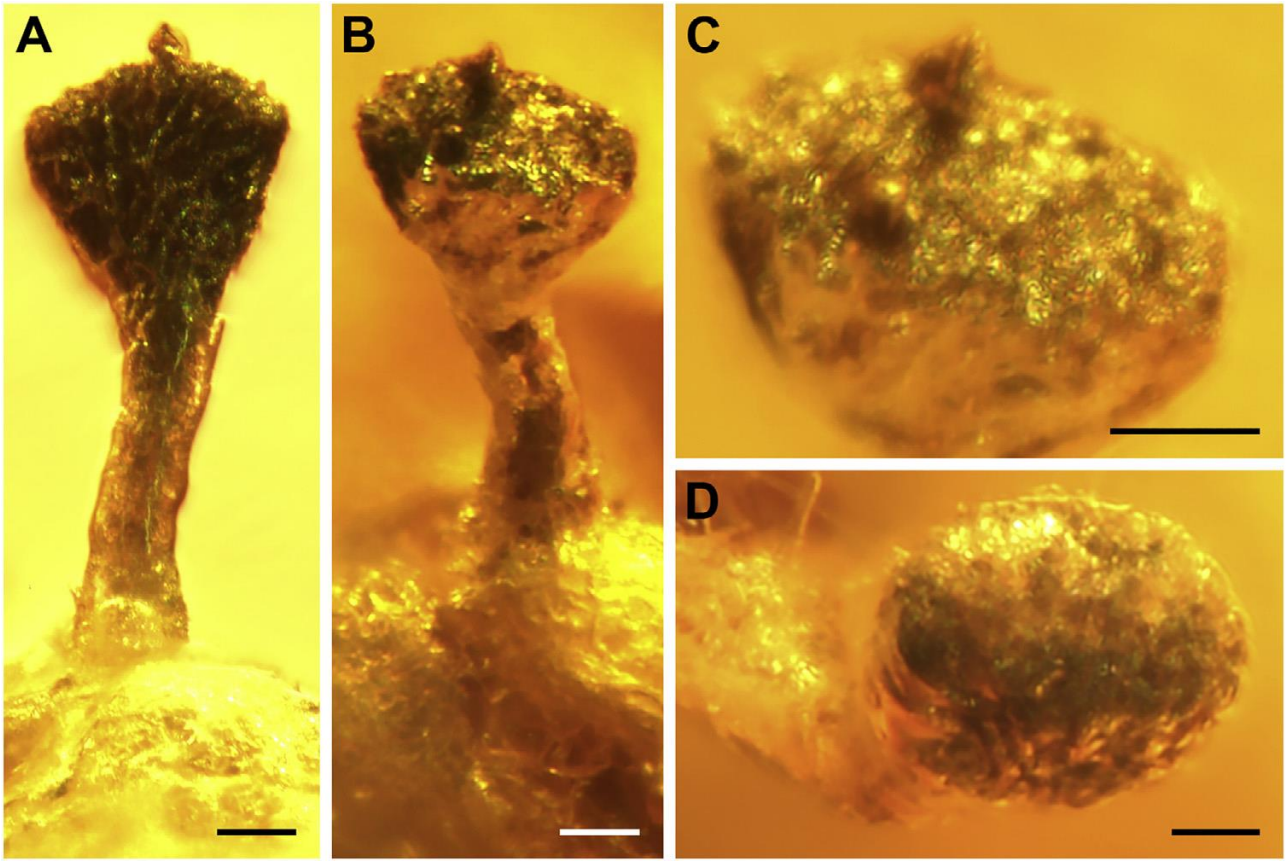


Fig. 7. Fossil *Phaeocalicium* specimen from Bitterfeld amber (GZG.BST.21960). A, B. Mature ascomata, note strongly compressed capitulum. C. Detail of capitulum surface with attached septate ascospores. D. Capitulum of immature ascoma. Scale bars: 20 mm.